

**REMARKS**

Claims 3, 5-29, 35 and 36 were pending in the application. Claims 3, 5, 8, 10, 15, 16, 20, 22 and 23 have been amended. Upon entry of these amendments, Claims 3, 5-29, 35 and 36 will be pending and under active consideration. Claims 3, 5, and 10 are independent.

Applicants submit respectfully that the amendments presented herein are supported fully by the claims and/or specification as originally filed and, thus, do not represent new subject matter.

Claims 3 and 5 have been amended to recite "one or both of" in place of "at least one of."

Claim 8 is amended herein to add the clarifying recitation, "at least one fluorescent dye is incorporated into each bound probe in each subset, but wherein each subset of bound probes incorporates a distinctly different amount of fluorescent dye" as suggested by the Examiner.

Claims 10, 15, 16, 20, 22, 23, 25 and 26 have been amended to more clearly state their scope and meaning as required by the Examiner. No new matter was added.

Claim 23 has been amended to point out more particularly and claim more distinctly that which Applicants regard as their invention by now reciting "oligonucleotides" instead of "oligonucleotide probes" as suggested by the Examiner.

Applicants wish to take this opportunity to thank Examiner for Examiner's withdrawal, noted at paragraph 1 of the Final Office Action, of the non-reiterated rejections and objections from the previous Office Action.

Applicants respectfully request entry of the amendments and remarks made herein into the file history of the present invention. Reconsideration and withdrawal of the rejections set forth in the above-identified Final Office Action are respectfully requested.

**I. The Rejections Under 35 U.S.C. § 102(b) Should Be Withdrawn**

The Final Office Action, at pages 10-17, paragraph 2, rejects Claims 3, 5, 7, 9-13, 15-27, 29, 35, and 36 as allegedly being anticipated by U.S. Patent No. 6,355,431 to Chee *et al.* (hereinafter, "Chee"), under 35 U.S.C. § 102(e). In sum, the Final Office Action alleges that Chee discloses an OLA (oligo ligation assay) reaction using first and second primers, including spectrally-addressable primers, hybridized to a target sequence and ligated with a thermostable ligase. In particular, the Final Office Action alleges further that Figs. 7A-F of Chee discloses the inventions of Claims 3, 5, 9-12, and 15-21. Applicants traverse respectfully.

Applicants submit respectfully that Claims 3, 5, 7, 9-13, 15-27, 29, 35, and 36, as amended, are not anticipated by Chee because Chee does not disclose each and every element of those amended claims as is required for a *prima facie* showing of anticipation. In particular, Claims 3 and 5, as amended, are directed to a nucleic acid ligation assay whereby target sequences that have the same nucleotide sequence in one portion but a different nucleotide sequence in another portion (*i.e.*, alleles of a gene) may be detected using a combination of free and bound probes wherein subsets of either the free or bound probes are substantially identical (*i.e.*, both bind to the portion of the target sequence that is the same) and other subsets of either the free or

bound probes are different (*i.e.*, only one of the subsets binds to the portion of the target sequence that is different; in other words, each subset binds to a different allele of the target gene). In either case, one free probe and one bound probe hybridize to the first and second target sequences, which are adjacent as shown in Figs. 1A and 1B, and are ligated for detection. Applicants submit respectfully that Chee does not teach or suggest the use such primers. The assays of Chee are directed solely at identifying a single target sequence.

The Final Office Action points to Column 3, lines 47-57, which disclose the use of a second primer binding to a second target sequence complementary to the first target sequence. The Final Office Action cites this passage as an example of identifying multiple target sequences. The Final Office Action notes, correctly, that complementary sequences are different. However, the Final Office Action alleges that, since these primers **can** attach to microspheres, they are considered as two subsets of bound primers. Without acquiescing in the allegation that both primers may be bound to microspheres, Applicant submits respectfully that both primers are not disclosed as **being** bound to microspheres; accordingly they do not teach two subsets of bound probes.

Furthermore, the Final Office Action alleges that primers that are complementary to each other are substantially identical. Applicant submits respectfully that one skilled in the art would recognize that complementary sequences are, by definition, completely different from each other except for rare palindromic sequences. The Final Office Action alleges that Applicant's definition of "substantially identical" sequences allows that such sequences may contain some differences. However, as the Examiner admits

at line 5 of page 12 of the Final Office Action, substantially identical nucleotides bind to the same sequence. By definition, complementary sequences bind each other, not the same sequence (except in the case of palindromic sequences, such as restriction sites). Accordingly, complementary sequences cannot be substantially identical.

In view of the above, Applicant submits respectfully that Claims 3 and 5, and their dependent claims, cannot be anticipated by Chee. Furthermore, with respect to Claim 9, the Final Office Action alleges that amplification of the target sequence by RCA (rolling circle amplification) anticipates the PCR amplification of Claim 9. One skilled in the art will recognize immediately that RCA and PCR are two entirely different processes. Also, the Final Office Action suggests that the thermostable ligase claimed in Claim 7 is anticipated by the ligase used in Chee's OLA because the ligation reaction is performed at a temperature that optimizes the activity of the ligase. One skilled in the art will recognize immediately that a thermostable ligase is one that can withstand temperatures approaching or exceeding the 100 degrees Celsius, as can the thermostable polymerases disclosed by Chee, not a ligase that has an optimal temperature. Chee does not disclose thermostable ligase. With regard to Claims 35 and 36, the Final Office Action alleges that Chee discloses microspheres with modifier moieties comprising primary amino groups. However, Claims 35 and 36 actually claim that the amine modifier is present on the oligonucleotide probe of the bound probe, not on the microsphere. Likewise, Claim 10 is not anticipated by Chee as Chee does not disclose the modifier moiety at one end of the oligonucleotide probe of the bound probe; accordingly, none of the claims dependent from Claim 10 can be anticipated. Therefore, Applicant submits respectfully that Chee does not anticipate any of these claims.

In sum, Applicant submits respectfully that the claims of the present invention, as amended, are not anticipated by Chee. Chee discloses a process that is wholly different from the processes claimed by Applicant. Chee discloses identifying a single target sequence and, sometimes, its complement. The complement is identified solely for purposes of amplifying the primary target sequence, and therefore cannot be considered to be a means of identifying a second target sequence different from the first sequence. Accordingly, Applicant requests respectfully that the rejection to Claims 3, 5, 7, 9-13, 15-27, 29, 35, and 36 under 35 U.S.C. § 102(b) be withdrawn.

**II. The Rejections Under 35 U.S.C. § 112, Second Paragraph, Should Be Withdrawn**

At paragraph 7 of the Final Office Action, Claims 3 and 5 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly and claim distinctly the subject matter regarded as the invention. The Final Office Action alleges that the recitation "at least one of" makes the claims unclear, and that deleting that recitation will make the claims more clear. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claims 3 and 5 are amended herein to recite "one or both of" in place of "at least one of" in order to clarify the scope and meaning of the claims. While the Final Office Action states that deletion of "at least one of" makes the claims clear and makes a better English sentence, Applicant submits respectfully that the claims do not have the same scope or meaning in the absence of such a

phrase. Since the remainder of the sentence is stated in the alternative (*i.e.*, detecting the presence **or** analyzing the nucleic acid sequence), only the practice of one of the alternatives at a time is claimed in the absence of omitted phrase. Accordingly, Applicant submits respectfully that an expression specifying that one or both of the alternatives may be practiced is needed to provide the full claim scope Applicant desires. Applicant submits respectfully that the expression “one or both” is a clear and unambiguous statement of the true scope of Applicant’s invention.

At paragraphs 8 and 9 of the Final Office Action, Claims 3 and 5 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly and claim distinctly the subject matter regarded as the invention. The Final Office Action alleges that the beginning and end of each claim do not correspond to each other. The Final Office Action alleges that the beginning of each of the claims recites, variously, only one target nucleic acid, free probe, or bound probe, whereas the end of each of the claims recites, variously, two target nucleic acids, free probes, or bound probes. Applicants traverse respectfully.

Applicant directs Examiner’s attention to the first paragraph following the preamble of each of Claims 3 and 5, wherein the claims recite “**one or more** target nucleic acid sequences with **one or more** subsets of free probes and **one or more** subsets of spectrally-addressable bound probes” (emphasis added). In view of the claims’ recitation, Applicant submits respectfully that the Final Office Action is in error with regard to the allegations of paragraphs 8 and 9.

At paragraph 10 of the Final Office Action, Claim 6 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly

and claim distinctly the subject matter regarded as the invention. The Final Office Action alleges that Claim 6 does not correspond to Claim 5 because Claim 5 requires that "the sample" is contacted with two subsets of free probes, whereas Claim 6 only requires that "a portion" of the sample contacts either the first or second subsets of free probes. Applicants traverse respectfully.

Respectfully, Applicant directs the Examiner's attention to the full recitation of Claim 6, which recites that "the assay is performed **in a first and a second reaction vessel**" and that **both** "a portion of the sample is contacted with the first subset of free probes in the first reaction vessel **and** a portion of the sample is contacted with the second subset of free probes in the second reaction vessel" (emphasis added). Accordingly, one skilled in the art would recognize that the sample is split into two portions, each of which is placed in a different reaction vessel and contacted with one of the two recited subsets of free probes. Accordingly, the sample as a whole is contacted by both subsets of free probes.

At paragraph 11 of the Final Office Action, Claim 8 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly and claim distinctly the subject matter regarded as the invention. The Final Office Action alleges that it is unclear how different subsets of probes may be distinguished as each uses the same fluorescent dye(s). Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claim 8 is amended herein to add the clarifying recitation, "at least one fluorescent dye is incorporated into each bound probe in a each subset, but wherein each subset of bound probes incorporates a distinctly different

amount of fluorescent dye." As amended, Claim 8 more clearly defines the method of distinguishing between different subsets of bound probes, the method being based on the relative amount of dye incorporated into the various subsets. Even though the same dye(s) is used in each subset, it is well known in the art that subsets of particles may be distinguished one from another when each subset of particles contains a different amount of dye and thus emits a different intensity of fluorescence. Accordingly, Applicant submits that Claim 8 is now clear and unambiguous in its scope and meaning.

At paragraphs 12 and 13 of the Final Office Action, Claim 10 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the reasons of record. In sum, the Final Office Action alleges that Claim 10 requires that two microspheres be bound to a bound probe that further comprises a modifier moiety. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claim 10, part (ii), is amended herein to recite "the bound probes comprise a microsphere and an oligonucleotide probe, wherein the oligonucleotide probes of a given subset of bound probes further comprise an oligonucleotide at one of their ends having a modifier moiety, which is used for coupling the oligonucleotide probe to the microsphere, and wherein the oligonucleotide probe further comprises an oligonucleotide having a predetermined nucleotide sequence that is complementary to at least another portion of the target nucleic acid molecules." Accordingly, Claim 10 is now clear and unambiguous in its meaning that the coupling moiety is used to couple the oligonucleotide probe of a bound probe to the microsphere of the same bound probe, not to a second microsphere. Further, it is also now clear



and unambiguous that the bound probes of each subset of bound probes are distinguishable from bound probes of other subsets by the unique spectral address exhibited by the microspheres of the various subsets.

At paragraph 14 of the Final Office Action, Claim 15 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly and claim distinctly the subject matter regarded as the invention. The Final Office Action alleges that Claim 15 does not correspond to Claim 10. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application Claim 15 is amended to recites that “the mixture comprises at least two subsets of bound probes, the oligonucleotide probes coupled to the microspheres of one subset being different from those coupled to the microspheres of the other subset.” Thus Applicant submits that Claim 15 now corresponds to Claim 10.

At paragraph 15 of the Final Office Action, Claim 16 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the reasons of record. In sum, the Final Office Action alleges that the language of Claim 16 is unclear. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claim 16 is amended herein to make its meaning and scope more clear. While Applicant notes that the Examiner's stated understanding of the intention of Claim 16 is correct, Applicant adds the clarifying expression

"oligonucleotide probes of" to the description of the various subsets. Accordingly, Applicant submits that Claim 16 is now clear and unambiguous.

At paragraph 16 of the Final Office Action, Claim 20 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the reasons of record.

Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claim 20 is amended herein to make its meaning and scope more clear. Applicant adds the clarifying expression "free probes" to the description of the various sets. Accordingly, Applicant submits that Claim 16 is now clear and unambiguous in view of the amended recitation of Claim 10 (from which Claim 11 depends) that "the free probes of a given set comprise two opposing ends, with a detectable label at one of their ends and a nucleotide at the opposite end."

At paragraph 17 and 18 of the Final Office Action, Claims 20 and 24 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for reciting the expression "at least two" when the base claims from which they depend recite "at least one." Applicants traverse respectfully.

Respectfully, Applicant submits that the allegations of the Final Office Action are in error. The expression "at least one set" would be understood by one skilled in the art as meaning "one or more sets." Likewise, "at least two sets" would be understood to mean "two or more sets." Accordingly, the dependent Claims 20 and 24 properly limit their base claims, as "two or more" is a proper subset of "one or more."

At paragraph 19 of the Final Office Action, Claims 25 and 26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for allegedly failing to have complete sentences. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claims 25 and 26 are amended herein to add the clarifying recitation, "wherein the assay is carried out." Accordingly, each of the claims is a complete sentence with a subject and a verb modifying the subject.

At paragraph 20 of the Final Office Action, Claim 28 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for allegedly failing to have complete sentences. Applicants traverse respectfully.

Without acquiescing in the propriety of rejection, and solely to advance prosecution of the present application, Claim 10 is amended herein to add the clarifying recitation, "or unique fluorescence intensity." Support for the amendment is found throughout the specification and claims as filed, for example at page 9, last full sentence, and Claims 8, 27 and 28 as filed. Accordingly, Claim 10 provides proper antecedent basis for the use of varying fluorescence intensities as a means of distinguishing populations, or subsets, of bound probes one from the other. Applicant notes that even spectrally-addressable microspheres, using the definition of spectrally-addressable as interpreted by the Examiner, may be further distinguished by fluorescence intensity.

On this basis, Applicants suggest respectfully that the rejections have been overcome, and Applicants request respectfully that the 35 U.S.C. § 112, second paragraph, rejection of Claims 3, 5, 6, 8, 10, 15, 16, 20, 24-26, and 28 be withdrawn.

**III. The Objections To The Specification And Claims Should Be  
Withdrawn**

At page 2, paragraphs 2-4, the Final Office Action objects to the specification and claims. At paragraph 2, the Final Office Action objects to Claims 3 and 5 due to the alleged informality that the “contacting step” recited at the beginning of each of the claims is identical to the contacting step in the last paragraph of each of the claims. At paragraph 3, the Final Office Action objects to Claim 23 for using the term “oligonucleotide probes” when the term “oligonucleotides” would allegedly be appropriate in light of Claim 10. Paragraph 4 notes that the substitute specification filed previously by Applicant was not entered because a clean copy of the specification, as amended, was not provided. Applicant traverses respectfully.

With regard to the allegations of paragraph 2, Applicant submits respectfully that the “contacting step” recited at the beginning of each of the claims is **not** identical to the contacting step in the last paragraph of each of the claims. In each case, the contacting step at the beginning of the claim is recited as part of a general description of the method of claim, and the second recitation of the contacting step is a more precise description of the contacting step as applied particularly to each claim.

For example, in Claim 3, the last paragraph limits the general contacting step at the beginning of the paragraph to particular conditions wherein each of the target nucleic acid sequences have different sequences in their first portion, but substantially identical sequences in their second portion. Accordingly, in this example, the spectrally-addressable bound probes have different sequences one from another, but

the free probes have substantially identical nucleotide sequences. The general description would allow the free probes to have different sequences, one from another. Conversely, in Claim 5, the later description of the contacting step provides that the spectrally-addressable bound probes substantially identical nucleotide sequences whereas the free probes have different sequences, one from another. The general description would allow the bound probes to have different sequences, one from another.

In view of the above, Applicant requests respectfully that the objection to Claims 3 and 5 be withdrawn.

With regard to the allegation of paragraph 3, Applicants herein amend Claim 23 to recite "oligonucleotides" rather than "oligonucleotide probes" in accordance with the Examiner's suggestion. Accordingly, Claim 23 conforms with Claim 10, as amended, and Applicant requests respectfully that the objection to Claim 23 be withdrawn.

With regard to the allegation of paragraph 4, Applicant submits herewith a clean copy of the specification as amended, in compliance with 37 C.F.R. 1.125 (b) and (c). Applicant apologizes for the inadvertent omission of a clean copy from Applicant's previous response, wherein only a marked-up copy was supplied. Accordingly, Applicant requests respectfully that the objection to the specification be withdrawn.

**CONCLUSION**

Applicant submits respectfully that the present application is in condition for allowance. Favorable reconsideration, withdrawal of the rejections set forth in the above-noted Office Action, and an early Notice of Allowance are requested.

Applicant's undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 625-3500. All correspondence should be directed to our address given below.

**AUTHORIZATION**

Applicant believes there is no fee due in connection with this filing. However, to the extent required, the Commissioner is hereby authorized to charge any fees due in connection with this filing to Deposit Account 50-1710 or credit any overpayment to same.

Respectfully submitted,



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